Title: METHOD FOR DETECTING PATHOGENIC MYCOBACTERIA IN

CLINICAL SPECIMENS



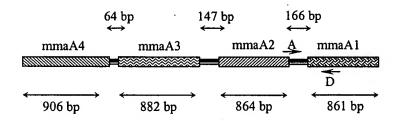


Fig. 1. Schematic diagram of methoxy mycolic acid synthase mmaA 4-mmaA 1 gene cluster of mycobacteria and location of forward A, and reverse D primers.

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REPLACEMENT SHEET

CTACTTCGCCAGCGTGAACTGGTTGACGTCGATGTAGCCGACCCGGAACAGCTTGGCGCAGCCGGTCA GGTATTTCATGTACCGCTCGTAGACCTCTTCGGACTGGATCGCGATGGCCTCGCTTTTGTGTTCCTGCA GCGCCTCGGCCCACAGGTCGAGGGTCCTGGCGTAATGCGGCTGCAGCGACTGGCGGCGAGTCAGCGT GAAACCCGTCTTCGCCGACTGTTCCTCAACCATTTCAATCGTCGGAGGTTGGCCCCCCGGGAAGATTTC GGTCGCGATGAACTTGAGAAAGCGGGCCAGCCACAACGTGAGCGGCAAGCCGTGGTCGACCATCTGC TGCCTGGTCAGGCCGGTGATCGTGCAGCAGCAACACGCCATCGGGCGGCAGGATTTTGTGGGCCCG GGCGAAGAAGTCGGCGTGACGATCGTGGCCGAAGTGCTCGAACGCGCCGATCGACACGATGCGGTCG ACGGGCTCGTTGAACTGCTCCCATCCCGCCAGCAACACTCGCCTGTCGCGGGGGTGTCCATCTCGTCG AACGACTTCTGCACATGGGCGGCCTGGTTCTTCGACAATGTCAGGCCGACGACGTTGACGTCATACTG CGCGATCGCGCGCATGGTGGCGCCCCAGCCGCAACCGATATCGAGCAGCGTCATGCCGGGCTGCA GACCTAGCTTGCCCAGCGCCAGGTCGATCTTGGCGATCTGGGCCTCTTCCAGCGTCATGTCCTCGCGTT CGAAATGCGCGCAGCTGTAGGTCTGGGTCGGATCCAGGAACAGCCGGAAGAAGTCGTCGGACAGGTC GTAGTGTGCCTGCACGTCCTCGAAGTGCGGCGTTAGGTCGTTGACCATgaggtgtaatgcctttccggaccctaggtggcct tteggtgcttgcaeggaaegcaecgatgetteccectecegcatgetcgaggcatgetatecgataeagggeegcegcactaaaecgcgategaatttgceeaggtca ${\tt gggaacggatatgagcggacgagCTACTTGGTCATGGTGAACTGGGCGACGTTGATTAGGCCTCTGCGGAAGCGCT}$ CCGCGCATCCGGTCAGATAGTGCATGAAGTTGTTGTAGACCTCTTCGGACTGTACGGCGATGGCGCGT TCGCGGGCAGCCTGTAGGTTGGCGGCCCATGCATCGAGAGTCCGTGCGTAGTGCTGCTGCAGCAGCTGG ACATGCTCGATGGTGAAGCCCGCGGCCTGCGCATTGTCGACAATGTCGGGCTCCGATGGCAGCTCGCC GCCCGGGAAGATCGACTCCCGCAGGAATTTGAGGAATCGAAGGTCGCTCATCGTCAGCGCAATGCCCT GTTCGTGCAGCCACCTGCGGTCGTAGGTGAACAGGCTGTGCAGTAGCATCCGCCCGTCATCGGGCAGG ATGTCGTAGGAGCGTTCGAAGAACGTCAGATACCGCTCCTTTTTGAACGCGTCGAATGCCTCAAAGCT TCCGATTGCGGCCAGGCGTCTTTGCTGCGTTCATAGTGATTCCGGCTGAGCGTGAGGCCGATGACATT GACGTCGTACTTCTCCACGGCCCGAACGAGCGCCCCGCCCCACCCGCAACCCACGTCGAGTAGCGTCA TCCCCGGTTCGAGGTTCAGCTTGTCCAACGCCAGATCCACCTTGGCCAGTTGCGCCTCTTCCAGCGTCA TATCGTCACGCTCGAAATAGGCGCAGGTGTAGACCCAGGTGGGATCGAGGAACAACGCGAAGAAGTC ATCCGAAATGTCGTAAGCCGACTGTGACTCTTCGTAATATGGTCTCAGCTTGGCCAT

Fig. 2. Sequence of mmaA2 and mmaA1 gene with an intergenic region of 166 base pair (shown in lower case. Location of forward A, sequence ID 1 and reverse primer D, sequence ID 2. Both primer sequences are underlined and italicized.

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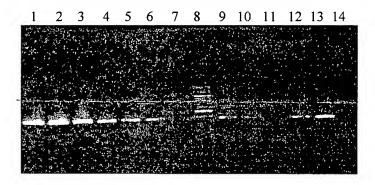


Fig. 3. PCR amplification of different mycobacterial genomic DNAs with primers A and D (lanes 1- 15): 1. *M.avium* 2. *M.bovis* 3. *M.chelonae* 4. *M.fortuitum* 5. *M.intracellulare* 6. *M.kansassi* 7. *M.phlei* 8. 100 bp DNA ladder 9. *M.marinum* 10. *M.scrofulaceum* 11. *M.smegmatis* 12. *M.szulgai*, 13. *M.tuberculosis* and 14. negative control. AD indicates 363 bp-amplified product.

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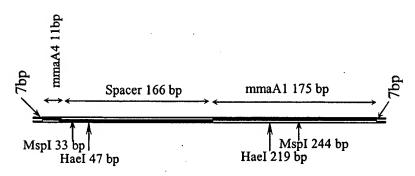


Fig. 4. Line diagram showing restriction endonuclease map of HaeI and MspI within AD.

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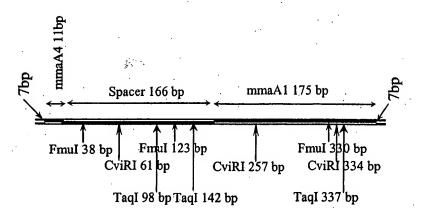


Fig. 5. Line diagram showing restriction endonuclease map of FmuI, CviRI and TaqI within AD.

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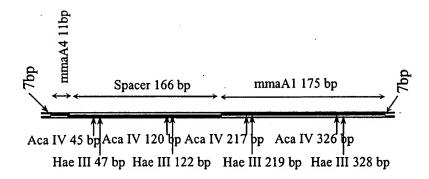


Fig. 6. Restriction map of AD showing distribution of the sites of restriction endonucleases AcaIV and HaeIII.

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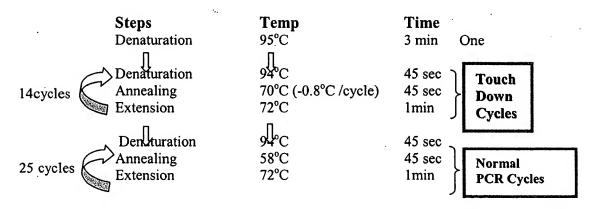


Fig. 7. Line diagram showing different steps of PCR reaction